Low-Dose Otelixizumab Anti-CD3 Monoclonal Antibody DEFEND-1 Study: Results of the Randomized Phase III Study in Recent-Onset Human Type 1 Diabetes

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OBJECTIVE

Previous studies demonstrated that the anti-CD3 monoclonal antibody otelixizumab, administered at a total dose of 48–64 mg, can slow the loss of C-peptide in recent-onset type 1 diabetes patients, with frequent reactivation of Epstein Barr virus (EBV). The DEFEND-1 (Durable Response Therapy Evaluation for Early or New-Onset Type 1 Diabetes) trial was designed to test whether a lower dose of otelixizumab could preserve C-peptide secretion in new-onset type 1 diabetes patients.

RESEARCH DESIGN AND METHODS

A multicenter, randomized, placebo-controlled trial was performed in sites in the U.S., Canada, and Europe. Two hundred eighty-one patients were randomized to treatment with 3.1 mg otelixizumab administered over 8 days or placebo. The primary end point of the study was the change in C-peptide area under the curve (AUC) from a 2-h mixed-meal tolerance test at month 12.

RESULTS

The change in 2-h C-peptide AUC was not different between placebo-treated patients and otelixizumab-treated patients (−0.20 vs. −0.22 nmol/L, $P = 0.81$). Secondary end points, including HbA_{1c}, glucose variability, and insulin dose, were also not statistically different between the two groups. More patients in the otelixizumab group than in the placebo group experienced adverse events, mostly grade 1 or grade 2. There was no EBV reactivation (viral load >10,000 copies/10^6 peripheral blood mononuclear cells) in the otelixizumab group, in contrast with previously published studies at higher doses of otelixizumab.

CONCLUSIONS

Otelixizumab was well tolerated in patients with recent-onset type 1 diabetes at a total dose of 3.1 mg, but did not achieve preservation of levels of C-peptide or other markers of metabolic control.

For patients with type 1 diabetes, the risk for the development of serious microvascular and macrovascular complications is proportional to the degree of chronic hyperglycemia, although these complications may remain subclinical during the pediatric and adolescent years (1). Among intensively treated patients participating

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**A complete list of investigators can be found in the Supplementary Data.*

in the Diabetes Control and Complications Trial, higher C-peptide concentrations (≥0.20 pmol/mL) at baseline were associated with a lower HbA_{1c} concentration, and a reduced risk for the development of diabetes complications and hypoglycemia (2). Therefore, therapies that preserve C-peptide concentrations above these levels may improve the outcome of patients with type 1 diabetes.

It is now widely recognized that type 1 diabetes reflects an autoimmune disturbance in which CD4+ and CD8+ T cells destroy insulin-producing β-cells in the pancreas in genetically susceptible individuals (3–5). Currently, insulin replacement therapy remains the principal treatment for type 1 diabetes (6), but achieving optimal glycemic control continues to be a persistent challenge (7). Insulin replacement therapy also fails to address the underlying disorder. With the discovery of several therapies that can change the progressive loss of insulin-producing β-cells, this treatment paradigm may be challenged. The potential of interdiction of the underlying autoimmune process to preserve β-cell function could facilitate glucose control, reduce long-term complications, and address the practical challenges of day-to-day disease management, which has a substantial impact on patients' quality of life.

Otelixizumab represents a novel, targeted, T-cell immunomodulator designed to induce long-term remission with a short course of therapy. Otelixizumab, a chimeric monoclonal antibody that targets the CD3/T-cell receptor, has been genetically modified to remove the glycosylation site in the Fc domain, thus diminishing complement or Fc receptor binding and reducing the risk of inflammatory adverse reactions secondary to cytokine release (8). Otelixizumab downregulates pathogenic T cells and upregulates T regulatory cells, thus inhibiting the autoimmune process responsible for type 1 diabetes (9). The potential utility of otelixizumab in the management of type 1 diabetes has been demonstrated in animal and human studies. In the nonobese diabetic (NOD) mouse model of spontaneous autoimmune diabetes, otelixizumab at a total dose of 8 μg yielded a 53% remission of diabetes, with as little as 30% CD3/T-cell receptor complex modulation, inducing a durable remission of diabetes (10). In this study, mice with greater residual β-cell function at the initiation of treatment were more likely to enter remission. In a phase II, double-blind, placebo-controlled study carried out by the Belgian Diabetes Registry (BDR), which included 80 patients with recent-onset type 1 diabetes, treatment with otelixizumab for 6 consecutive days, for a total dose of 48–64 mg, reduced insulin requirements and preserved β-cell function (11,12). Indeed, at 36 months, residual β-cell function was 80% higher in the otelixizumab group than in the placebo group. These beneficial effects were correlated with higher residual β-cell function at baseline and treatment at a younger age (11).

The Durable Response Therapy Evaluation for Early or New-Onset Type 1 Diabetes (DEFEND-1) study was a randomized, placebo-controlled, multinational, phase III trial designed to evaluate the efficacy and safety of otelixizumab after a single course of treatment in subjects with new-onset type 1 diabetes. A lower dose than the phase II study was chosen in order to target a lower rate of Epstein Barr virus (EBV) reactivation than seen in the phase II study (75%) (12). The primary outcome analysis of the DEFEND-1 trial was to compare the change from the baseline C-peptide area under the curve (AUC) at month 12 between the otelixizumab group and the placebo group (13,14). The large demographic data set from this study afforded an opportunity to investigate the relationship between fasting and stimulated C-peptide levels as well as the impact of optimal glycemic control on C-peptide levels.

RESEARCH DESIGN AND METHODS

DEFEND-1 Design

DEFEND-1 was a randomized, placebo-controlled, double-blind, multicenter study conducted in the U.S., Canada, Germany, Denmark, Spain, Finland, the U.K., Italy, and Sweden. Subjects provided consent to participate under approved protocols from local institutional review boards and were randomized in a 2:1 ratio to receive treatment with otelixizumab or placebo. Subjects received a series of eight intravenous infusions—one infusion per day over 8 consecutive days. The doses of otelixizumab administered were 0.1 mg on day 1; 0.2 mg on day 2; 0.3 mg on day 3; and 0.5 mg/day on days 4–8, for a total dose of 3.1 mg. According to each investigator's clinical judgment, prophylaxis or treatment for cytokine release syndrome was administered each dosing day, 2 h before the study drug or placebo infusion, right after the infusion, 6 h after the infusion, and at bedtime. Medications included ibuprofen 400–800 mg to a maximum of 1,800 mg/24 h or acetaminophen 500–1,000 mg to a maximum of 4,000 mg/24 h, and diphenhydramine 25–50 mg or other equivalent antihistamine.

At key study visits (screening, predose [≤14 days], week 12, month 6, and month 12), all subjects underwent a mixed-meal tolerance test (MMTT) with Boost. If the time from screening to randomization was >35 days, the predose MMTT was used as the baseline measurement. Fasting C-peptide level was measured prior to the meal, and the maximum stimulated C-peptide level was the maximum observed C-peptide level after the meal. Blood samples for the measurement of C-peptide secretion were taken at the following intervals in adults: 10 min before time 0 (−10 min); immediately before the subject started drinking Boost (designated as the time 0 sample); and 15, 30, 60, 90, and 120 min after time 0. For adolescent subjects (<18 years of age) at the time of screening, blood samples were taken at the 120 min time point only; at subsequent visits, blood samples were taken at all time points. The C-peptide AUC was calculated. The mean daily insulin dose was computed as the mean of the subject's daily total insulin use recordings (in international units [IU] per kilogram) over a 7-day period, within 14 days of study visits, the predose MMTT, and prior to the study drug.

Vials of the study drug were sent to the study centers under blinded conditions. Because the person who prepared the study drug solutions could become unblinded as a result of close observation of the material, that person was not involved in any other aspect of the study. The investigator and other personnel who administered the study drug and/or performed study assessments did not watch or participate in the preparation of the study drug. Inadvertent unblinding can occur as a result of the cytokine release signs and symptoms

experienced by some subjects who receive otelixizumab, and the transient decreases in lymphocyte counts that are expected to occur. Investigators, other study center staff, and the study sponsor were blinded to laboratory data such as lymphocyte counts during the infusion period, to reduce the possibility of unblinding. An unblinded independent medical monitor did have access to such laboratory data. Although the unblinded independent medical monitor did not know subject treatment group assignments, that person could potentially become unblinded by reviewing data. During the first 12 months after administration of the study drug, study clinic visits occurred frequently (weekly to monthly).

Inclusion Criteria

Male or female subjects were 12–45 years of age with a diagnosis of new-onset (≤90 days between the initial diagnosis and the first dose of study drug) type 1a autoimmune diabetes based on American Diabetes Association and World Health Organization criteria. All subjects required insulin treatment currently or at some point between the date of diagnosis and administration of the first dose of the study drug. Subjects were positive for one or more of the following autoantibodies typically associated with type 1 diabetes: glutamic acid decarboxylase; tyrosine phosphatase–like protein; zinc transporter autoantibodies; or insulin, if using insulin for <7 days. Subjects demonstrated evidence of residual functioning β-cells as measured by a stimulated C-peptide level of >0.20 nmol/L during an MMTT when fasting blood glucose levels were >70 and ≤200 mg/dL, with a maximum stimulated C-peptide level of ≤3.50 nmol/L. Subjects ≥18 years of age at screening were required to have a BMI of <32 kg/m^2. For subjects <18 years of age at screening, the BMI was required to be in the <95th percentile for age and sex, according to U.S. Centers for Disease Control and Prevention (15). Subjects needed to be in generally good health, with no significant and/or active disease in any body system and no clinically significant abnormal laboratory values.

Objectives

Using DEFEND-1 baseline data, the post-intervention results compared mixed-meal–stimulated and fasting C-peptide levels, and differences by age group for these and other metabolic variables in adults and adolescents. Using baseline data, glucose variability was evaluated in relationship to C-peptide level. The primary efficacy end point was to compare the change from baseline in the stimulated serum C-peptide level (mean AUC over the 2-h period after an MMTT) between the otelixizumab group and the placebo group at 12 months (16). Secondary efficacy variables included mean total daily insulin use over 7 consecutive days, summarized at baseline and key visits, and HbA_{1c} level, insulin use, and insulin dose–adjusted HbA_{1c} level at 12 months. The following two measures of glucose variability were evaluated for the relationship to C-peptide: average daily risk range (ADRR) (17), and mean amplitude of glycemic excursions (MAGEs), both at 12 months (18).

Investigators encouraged subjects to get as close as possible to an HbA_{1c} concentration of 6%. All adverse events were recorded. Hypoglycemic events that were reported by subjects between study visits were classified, as defined by the American Diabetes Association and Food and Drug Administration guidelines, as follows: severe—requiring assistance of another person to actively administer carbohydrate, glucagon, or other resuscitative actions; documented—typical symptoms of hypoglycemia accompanied by a measured plasma glucose concentration of ≤3.9 mmol/L; and asymptomatic—not accompanied by typical symptoms of hypoglycemia but with a measured plasma glucose concentration of ≤3.9 mmol/L (19,20). Perturbation of EBV immunity was assessed by EBV DNA viral load (an EBV DNA viral load of >10,000 copies/10^6 peripheral blood mononuclear cells [PBMNCs] was considered to be above normal) each day from day 1 to day 8. If a subject had positive test results, the test was repeated weekly for 2 weeks or until the count decreased to <10,000 copies/10^6 PBMNCs (whichever time was longer). Serologic tests were also performed for antibodies to hepatitis B surface and core antigens. The lymphocyte subsets, CD3 saturation, and CD3 modulation were measured by flow cytometry, and data were analyzed using the FCS Express V3 Clinical Edition software. Antibodies that were used in flow cytometry experiments included anti-human Foxp3 and TcRalpha+TcRbeta (An Der Grub Bio Research GmbH). Antibodies from BD Bioscience included anti-human CD2 fluorescein isothiocyanate, CD2 phycoerythrin, CD25 phycoerythrin, CD8 peridinin chlorophyll, and CD4 allophycocyanin. C-peptide level was measured using the competitive IMMULITE C-Peptide chemiluminescent immunometric assay and an IMMULITE series analyzer.

Statistical Analyses

Demographic and other baseline characteristics were summarized using descriptive statistics. The intent-to-treat (ITT) population was used for efficacy and pharmacodynamic analyses. A mixed-model repeated-measures methodology was used with a "missing at random" assumption in which the missing data may depend on the prior observations (i.e., the conditional independence assumption). The model provides unbiased least-squares mean estimates for the treatment groups at the assessment time points. The per protocol (PP) population was defined as ITT subjects who had completed all scheduled infusions of the study drug and received the correct doses, and for whom there were recorded MMTT C-peptide data, with at least one pre-MMTT and at least two post-MMTT samples, at both baseline and month 12. The PP population also did not use a prohibited concomitant medication while in the study. The primary efficacy end point was analyzed using a repeated-measures mixed-effects model with change from baseline C-peptide AUC, age group, continent (North America or Europe), treatment group, visit, and treatment group by visit interaction as prespecified independent variables. Assumptions made in the power calculation were based on a comparable study carried out by the BDR, in which 80 subjects received either otelixizumab or placebo. In the BDR study, the observed difference between the otelixizumab and placebo groups in mean change from baseline to month 12 in C-peptide AUC was 0.22 (nmol/L × min)/min. Therefore, this was used as the assumed value for the clinically meaningful treatment effect for otelixizumab. With these assumptions, at a two-sided significance level of 0.05, a sample size of 180

subjects (120 in the otelixizumab group and 60 in the placebo group) provided 90% power to detect a treatment effect of 0.22 (nmol/L × min)/min on the primary efficacy end point with a two-sided *t* test. Assuming a drop-out rate of 25%, the total planned sample size for the study was 240 subjects (160 in the otelixizumab group and 80 in the placebo group). In addition to the repeated-measures analysis, ANCOVA of the change from baseline in C-peptide AUC to month 12 was performed on the ITT population. The ANCOVA model contained terms for baseline C-peptide AUC, continent, age, and treatment. In the ITT analysis, missing month 12 data were imputed using the last observation carried forward.

A subject was considered a responder if, at a given visit, the subject had an HbA_{1c} concentration of ≤6.5% and mean total daily insulin use over 7 consecutive days of <0.5 IU/kg/day during the 2 weeks preceding the visit. Responders were compared by two-sided tests of equality based on the normal approximation to the binomial distribution; Hochberg-adjusted *P* values were reported. Exogenous insulin use and HbA_{1c} end points were compared using the same repeated-measures mixed-effects model as the primary end point. The composite outcomes HbA_{1c}/exogenous insulin use and C-peptide AUC/HbA_{1c}/exogenous insulin use were compared between treatments using the O'Brien nonparametric ranks test procedure.

Glucose Variability

Two measures of glucose variability were evaluated for the relationship to C-peptide: ADRR and MAGE. Spearman correlations were used to assess associations among C-peptide AUC, HbA_{1c} concentration, MAGE, and ADRR. Subjects were divided into tertiles based on C-peptide AUC to further explore the association between C-peptide level and other variables.

RESULTS

Screen Study Description

Three hundred eighty-one subjects were assessed for eligibility, and 272 subjects met the entry criteria. One hundred nine patients did not meet the eligibility criteria for a variety of reasons, including exclusions for BMI, unrelated laboratory abnormalities, and lack of proven autoimmunity, with the most common single reason being low C-peptide response. The 272 subjects were randomized to otelixizumab (*n* = 181) and placebo (*n* = 91) (Fig. 1). Data were analyzed for the ITT and PP populations. Since there were no significant differences between these two types of analyses, the ITT analysis is presented here.

Demographics of Enrolled Subjects

Subject demographics are noted in Table 1. No major baseline differences were noted among study subjects. Furthermore, there were no major differences noted among subjects from different countries. There were slight differences in the level of mean stimulated C-peptide level between the subgroup of adolescent subjects compared with adult subjects that were not clinically relevant (0.81 vs. 0.72 nmol/L/min, respectively). The prevalence of type 1 diabetes–associated antibodies was

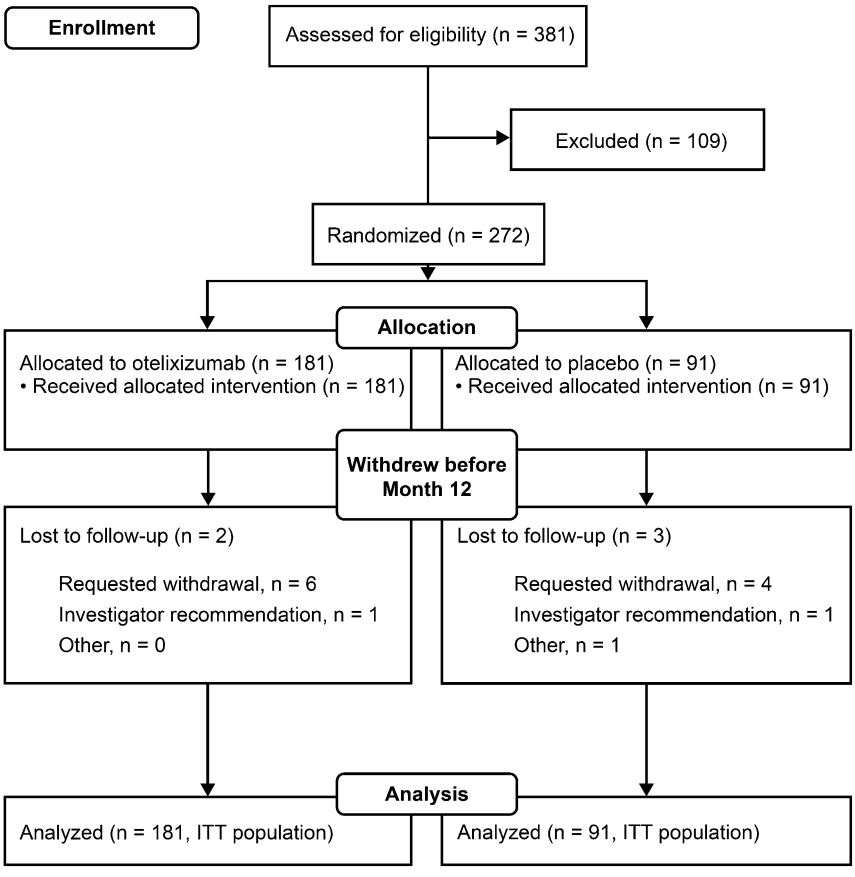


Figure 1—Flow chart: enrollment of the DEFEND study.

Table 1—Baseline characteristics

Characteristics	Placebo group (*n* = 91)	Otelixizumab group (*n* = 181)
Age, years	25.3 ± 7.2	24.8 ± 6.6
Sex, *n* (%)		
Female	31 (34.1)	64 (35.4)
Male	60 (65.9)	117 (64.6)
Ethnicity, *n* (%)		
Hispanic/Latin American	3 (3.3)	8 (4.4)
Non-Hispanic/Latin American	88 (96.7)	173 (95.6)
Race, *n* (%)		
American Indian or Alaska Native	0 (0)	1 (0.6)
Asian	2 (2.2)	1 (0.6)
African American	2 (2.2)	6 (3.3)
Multiracial	2 (2.2)	4 (2.2)
Caucasian	85 (93.4)	169 (93.4)
BMI, kg/m^2	23.6 ± 3.0	23.6 ± 3.3
Weight, kg	72.2 ± 12.2	71.9 ± 14.6
HbA_{1c}		
%	7.26 ± 1.55	7.25 ± 1.29
mmol/mol	56 ± 16.9	56 ± 14.1
Time-normalized stimulated C-peptide AUC, (nmol/L × min)/min*	0.70 ± 0.36	0.75 ± 0.42
Maximum stimulated C-peptide, nmol/L*	1.00 ± 0.51	1.03 ± 0.55

Data are the mean ± SD, unless otherwise specified. *Placebo *n* = 90; otelixizumab *n* = 179.

similar among all groups. More detailed information is shown in Table 1.

Two-hour MMTT AUC—Primary End Point

The primary end point was the change from baseline in 2-h C-peptide AUC from an MMTT at month 12. Using an ITT analysis, there was no difference noted between placebo-treated and drug-treated patients (−0.20 ± 0.037 vs. –0.22 ± 0.025 nmol/L, respectively; *P* = 0.58) (Fig. 2*A*). There was also no difference in the change from baseline in C-peptide level between placebo-treated and otelixizumab-treated patients after adjustment for age, continent, and baseline C-peptide level (−0.21 ± 0.030 vs. −0.21 ± 0.021 nmol/L, respectively; *P* = 0.81). Prespecified subgroup analyses of baseline C-peptide AUC, adolescent and adult subjects, North American and European subjects, baseline insulin use, HbA_{1c} level, BMI, gender, and type and number of positive autoantibodies were performed; all failed to demonstrate a significant difference between the two groups (Supplementary Table 1).

Secondary End Points

There was no significant difference from baseline to month 12 between the placebo and otelixizumab groups in the secondary end points of mean HbA_{1c} level (6.91 ± 0.147% vs. 7.04 ± 0.116%, respectively; *P* = 0.289) (Fig. 2*B* and Supplementary Table 2) and mean daily insulin dose (0.43 ± 0.026 vs. 0.39 ± 0.017 IU/kg, respectively; *P* = 0.276) (Fig. 2*C* and Supplementary Table 2). There were no significant differences between the otelixizumab and placebo groups in other secondary end points, including ADRR and MAGE (Supplementary Table 2).

Adverse Events

Adverse events were more common in the otelixizumab group, and included, for example, headache, fever, rash, nausea, which were consistent with known side effects of anti-CD3 antibodies (Supplementary Table 3). No subjects in the otelixizumab arm had EBV, as determined by PCR results of >10,000 copies/10^6 PBMNCs, whereas in an earlier phase II trial (12), 75% of study subjects had symptomatic disease with reactivation of EBV after receiving 48–64 mg otelixizumab. Only one subject (an adult in the placebo group) had EBV, as determined by a PCR result of >10,000 copies/10^6 PBMNCs at weeks 6 and 12. The incidence of adverse events related to the perturbation of EBV immunity was similar between the treatment groups. Oropharyngeal pain was more frequently reported in the placebo group compared with the otelixizumab group. Although the incidence was low, pharyngitis and viral infection were more frequent in the placebo group compared with the otelixizumab group. Papular rash was reported only in the otelixizumab group, but maculopapular rash was reported in both groups with proportionately more in the placebo group.

Mechanistic Studies

Transient reductions in lymphocytes and modulation of CD3 T-cell receptor were observed during the dosing period in otelixizumab-treated patients (Fig. 3*A* and *B*, respectively), which is consistent with observations in previous studies investigating the same regimen (data not shown). However, the maximum reduction in lymphocytes (36.3% relative to predose values) was less than that observed during therapy in the phase II trial using otelixizumab (12) and in previous trials with teplizumab (21), another anti-CD3 monoclonal antibody. Anti-CD3 treatment in the NOD mouse has been shown to increase regulatory T-cell populations in addition to its other effects (22). We examined changes in the number of CD4+CD25+FoxP3+ T cells in those individuals treated with otelixizumab, and while, as expected, there was a transient reduction during the dosing period in otelixizumab-treated patients, no treatment differences were observed beyond day 14 (Supplementary Fig. 1).

CONCLUSIONS

The DEFEND-1 trial recruited a population that was representative of the type 1 diabetes population. Unfortunately, the cumulative dose chosen for this study, 3.1 mg otelixizumab, was not effective in preserving C-peptide level, as had been previously seen in the phase II study (11). Modulation of the CD3/T-cell receptor complex has been shown to be effective in preserving C-peptide levels in previous studies using both otelixizumab (11,12) and teplizumab, another chimeric/humanized anti-CD3 monoclonal antibody (21,23,24). However, it is important to take into account the relative dosages used in these individual clinical trials when conducting cross-study and cross-compound comparisons. The three otelixizumab efficacy studies,

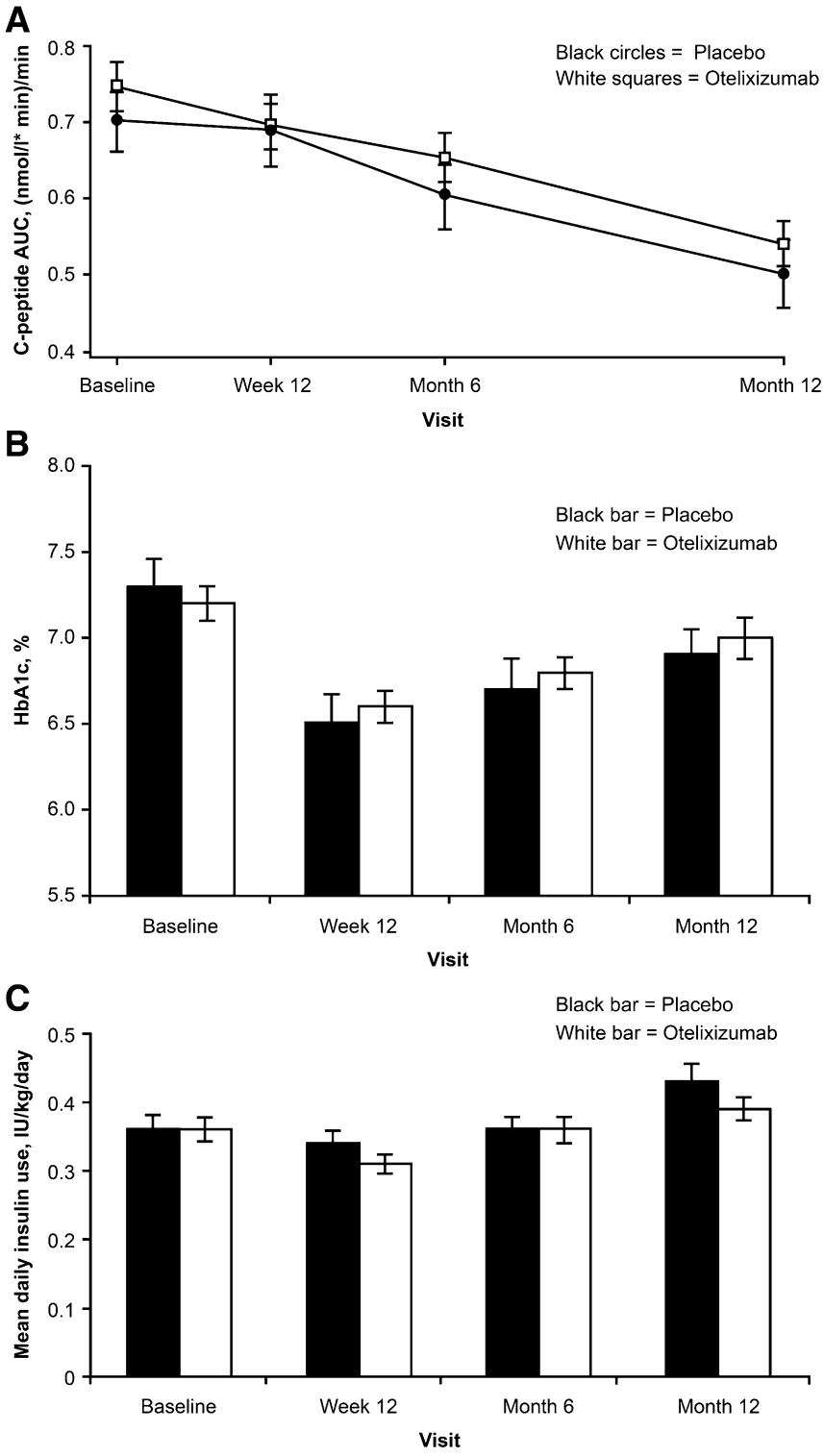
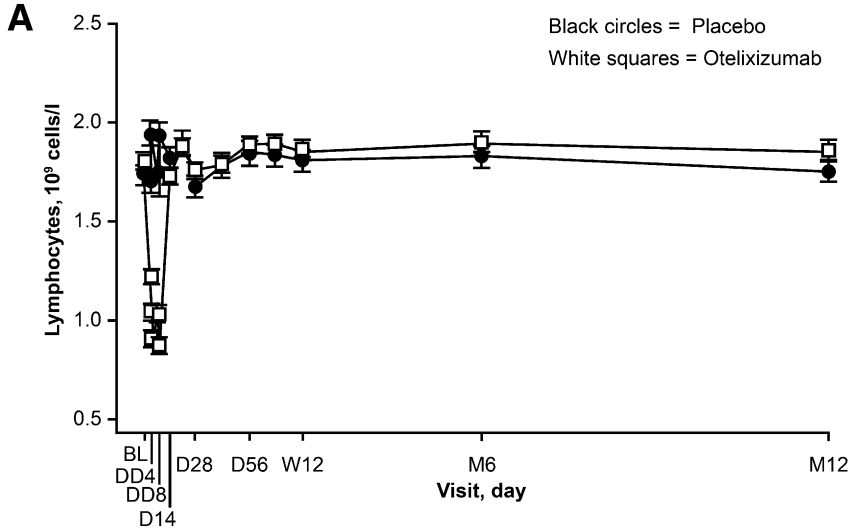


Figure 2—Comparison of otelixizumab vs. placebo for C-peptide mean AUC (*A*), HbA_{1c} levels (*B*), and insulin use over 12 months (*C*).

BDR (48 mg) (11), DEFEND-1, and subsequently Durable-Response Therapy Evaluation For Early or New-Onset Type 1 Diabetes (DEFEND-2) (25) (3.1 mg doses), had very different risk/benefit profiles. With respect to otelixizumab, at a dose of 48 mg, a sustained beneficial effect on C-peptide levels was seen, with a reduction in insulin dose for up to 4 years, although this was at the expense of severe symptoms of cytokine release syndrome and reactivation of EBV. At a dose of 3.1 mg, no efficacy was seen, although symptoms of cytokine release syndrome were significantly milder, and no reactivation of EBV was seen. Teplizumab delivered at a dose of 17 mg appears to deliver an intermediate efficacy response (positive effect on C-peptide levels at ~6 months), with an intermediate effect



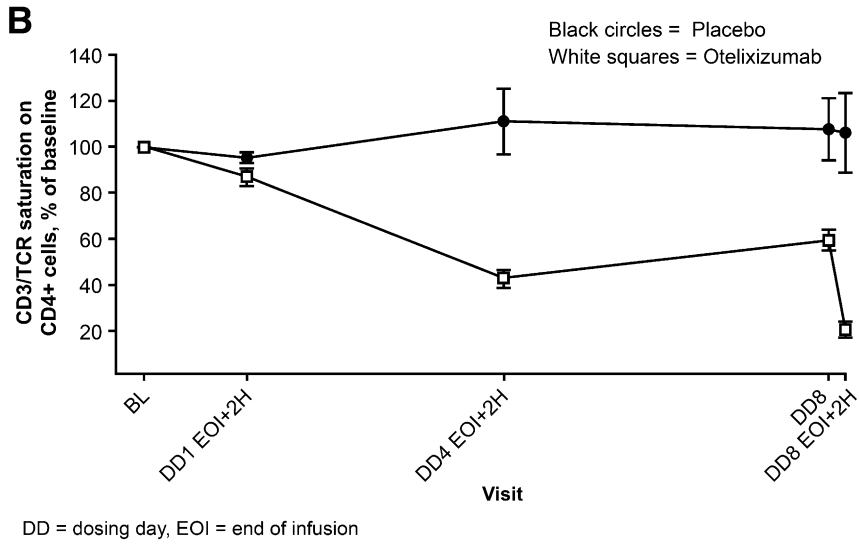


Figure 3—Lymphocyte depletion in otelixizumab-treated vs. placebo-treated patients (*A*) and modulation of CD3 on the cell surface (*B*). BL, baseline; D, day; DD, dosing day; EOI, end of infusion; H, hour; M, month; W, week; TCR, T-cell receptor.

on cytokine release syndrome symptoms (24). Therefore, it appears that if a sufficient dose of antibody is administered, a meaningful clinical effect can be observed. In this study, other factors, such as level of C-peptide at baseline, age, and sex, were examined to determine whether a cohort of subjects could be found that showed efficacy, but these subgroup analyses were also not statistically significant (Supplementary Table 2).

One measure of the pharmacological activity of anti-CD3 therapy is the relative level of T-cell lymphopenia observed in the peripheral blood. Although otelixizumab appears not to be a fully depleting antibody, based on animal studies, its administration causes the modulation of CD3/T-cell receptor complexes from the cell surface of T cells, and their subsequent transient redistribution from the bloodstream into tissues and lymph nodes (26). Relatively low levels of CD3 binding and downmodulation induced by short treatment of anti-CD3 Fab2 fragments in the NOD mouse led to lifelong reversal of established diabetes in this model of type 1 diabetes (10). In contrast, in the original clinical proof-of-concept study (BDR study), the administration of a 48- to 64-mg cumulative dose of otelixizumab resulted in an almost complete loss of circulating CD3+ T cells in patients for the duration of treatment, recovering to predose levels by week 3. The reasons for the lack of congruence between dosing of anti-CD3 therapy in the NOD mouse and in type 1 diabetes patients remains unclear, but may include species differences in peripheral blood versus target organ marker dynamics and/or differences in the pharmacologic behavior at the site of action of a Fab2 fragment versus an intact monoclonal antibody. Further preclinical and clinical studies will be needed to understand and improve the translatability of this model. Likewise, the relationship between the level of CD3 modulation or lymphopenia and efficacy has not been established clinically with otelixizumab. It appears that the dose administered in the BDR study

may be at the top of the dose response from the perspective of CD3 modulation at least, and data from clinical studies with teplizumab suggest that subsaturating doses of an anti-CD3 monoclonal antibody may be efficacious (21).

Dose exploration studies had been conducted prior to the start of the DEFEND-1 study but had a limited scope, primarily related to safety and tolerability, in patients with established disease and with no control or placebo group. While the regimen chosen was shown to be tolerable with respect to cytokine release syndrome and EBV reactivation, clinical evidence for the efficacy of the DEFEND-1 dose regimen was lacking. Further dose finding in new-onset diabetic patients is required to establish the efficacy and therapeutic window of otelixizumab.

Reactivation of latent herpes viruses is a common event in clinical studies with immunomodulating agents. Of particular concern is the reactivation of EBV, due to the oncogenic potential of this virus (27). It was felt that the level of EBV reactivation (identified as a syndrome similar to acute mononucleosis, manifesting with sore throat, fever, and cervical adenopathy between day 16 and day 21 after the first infusion) observed with otelixizumab in the original BDR study was unacceptably high, at 75% (12), and that a lower dose needed to be found that could avoid this complication. Of note, no significant reactivation of EBV (EBV DNA viral load $>$10,000 copies/10^6 PBMNCs) was noted with the dose of otelixizumab used in the DEFEND-1 trial. The severity of the adverse events in the present trial was, in general, milder than that seen in the phase II trial and was mostly grade 1 or 2. Furthermore, all symptoms connected with potential cytokine release were diminished with this dose, along with pretreatment of patients with ibuprofen or acetaminophen and antihistamines. Infections and other adverse events were more frequent in the otelixizumab group, but were not significant and were also generally mild (grade 1 and 2). Higher doses of otelixizumab could lead to better efficacy with still tolerable levels of adverse events and should be explored.

To date, trials of immunotherapy have suffered from the lack of a secondary immunologic end point that could guide dosing of therapy. Therapy with both anti-CD3 and anti-CD20 appears to be most effective when each of the drugs is administered at a dose that can markedly reduce the respective cell populations within the bloodstream (28,29). It may be that markers such as these, or hopefully ones that may be more disease specific, can be found to help guide dosing in future trials. In addition, the heterogeneity of type 1 diabetes in terms of age of onset, residual β-cell function, and HLA genotype should be taken into account in future trial designs (30). In conclusion, while a dose reduction of otelixizumab appears to effectively improve its safety profile, such a reduction sacrifices efficacy. Further dose-ranging studies should be undertaken to effectively determine a viable therapeutic window with proven efficacy and a tolerable safety profile.

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GSK has made the placebo and baseline data from the DEFEND studies available in a deidentified, patient-level format. They would be happy to receive proposals for secondary data analyses from external researchers once DEFEND-1 and DEFEND-2 primary publications are accepted for publication. Please contact Dr. Jill Donaldson (jill.x.donaldson@gsk.com) for further information.

Author Contributions. R.A., P.A.G., E.B., and P.P. participated as local Principal Investigators in the conducting of the trial, reviewed and edited the manuscript, were fully responsible for all content and editorial decisions, and were involved at all stages of manuscript development. J.S.C. contributed to data collection and interpretation of the results, reviewed and edited the manuscript, was fully responsible for all content and editorial decisions, and was involved at all stages of manuscript development. T.W.D. assisted in the design of the study, participated as a local Principal Investigator in the conducting of the trial, reviewed and edited the manuscript, was fully responsible for all content and editorial decisions, and was involved at all stages of manuscript development. B.W.B. participated in the recruitment and ongoing care of study subjects; participated in the formatting, editing, writing, and reviewing of the manuscript; was fully responsible for all content and editorial decisions; and was involved at all stages of manuscript development. R.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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